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A note on perfusion with labelled compounds to determine intestinal absorption

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An *in vivo* perfusion technique has been adapted for the measurement of intestinal absorption of labelled compounds. Salicylic acid, labelled with ^{14}C, in solution with or without phenylephrine or caffeine, was perfused through the ligated and cannulated small intestine *in situ* of the rat. Absorption was estimated from the difference in count of the solutions entering the gut and the outflow which was sampled at 10 min intervals. Some 40–45% of the acid was found to be absorbed at each of three consecutive 10 min samplings. This was lower than, but consistent with, reported results derived by chemical assay. In the presence of the other two drugs the absorption of salicylic acid increased to 70%.

SEVERAL *in vivo* procedures for determining intestinal absorption have been described (e.g. Cori, 1925; Sheff & Smyth, 1955; Nissem & Smith, 1965). Shanker, Tocco, Brodie & Hogben (1958) described a procedure for determining intestinal absorption in anaesthetised rats. Buffer solutions containing the drugs or compounds are perfused, at a rate which can be varied, through the intestine from the duodenal to ileal end by means of a perfusion pump. The ileal outflow is collected and the degree of absorption determined from the difference of concentrations of the compound entering and leaving the intestine as determined by chemical analysis. We have used this procedure, with suitable modifications, to measure the uptake of salicylic acid. This drug was chosen because of its wide use both as a single component and in combination with other drugs. The final analysis was made by radioisotope activity measurement.

Experimental

Preparation. Female Wistar rats, 80–100 g, were fasted for 24 hr and anaesthetised with pentobarbitone, 35 mg/kg, administered intraperitoneally. A midline incision was made in the abdominal region, the intestine exposed and cannulated at the duodenal and ileal ends with tygon cannulae having an inside diameter of 2·5 mm and outside diameter of 3·5 mm. The stomach and caecum were closed off by ligatures, taking care not to occlude major blood vessels. The intestine was replaced in the abdomen, the incision clamped and perfusion through the intact small intestine initiated immediately.

Perfusion procedure. Drug solution (300 ml) in a 400 ml Erlenmeyer flask was placed into a constant temperature water bath and maintained at 37°. The solutions were perfused through the intestine at a rate of 1·5 ml/min by means of a Sigmamotor Peristaltic pump (Sigmamotor, Inc., Model A–L–4–F, Middleport, N.Y.) The level of the solutions was such that flow through the preparation was not influenced by gravity or hydrostatic pressure.

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The perfusion rate was adjusted before each perfusion. The small intestine was cleared of particulate matter by perfusing for 30 min with drug-free solution and then for 30 min with drug solution to displace the first wash. Perfusion was continued for a further 30 min with collections of the ileal outflow in separate 10 min intervals. Three 0·1 ml samples of each 10 min collection were taken for counting.

The procedure was repeated with 5 animals for each drug solution. The drug solutions employed were as follows (mM/litre water). Solution 1. Salicylic acid, 1·0; NaCl, 145·0; KCl, 4·56; $CaCl_2$, 1·26; Na_2HPO_4, 1·33; NaH_2PO_4, 0·33. Solution 2. Salicylic acid, 1·0; phenylephrine HCl, 0·03; NaCl, 145·0; KCl, 4·56; $CaCl_2$, 1·25; Na_2HPO_4, 1·33; NaH_2PO_4, 0·33. Solution 3. Salicylic acid, 1·0; caffeine, 0·09; NaCl, 145·0; KCl, 4·56; $CaCl_2$, 1·25; Na_2HPO_4, 1·33; NaH_2PO_4, 0·33.

The ratio of phenylephrine and caffeine to salicylic acid was similar to that in commercial preparations. For the study, ^{14}C-carboxyl labelled salicylic acid was added to each solution, 5 μc/300 ml, and each solution thoroughly agitated to give a homogeneous mixture. The radiochemical purity of the labelled salicylic acid was established by paper partition chromatography and autoradiography.

Counting procedure. The 0·1 ml volumes of perfusate obtained from each animal at the various time intervals were added to 10 ml of a liquid scintillation solvent system in glass counting vials [diphenyloxazole 0·4%, naphthalene 5·0%, cellosolve 300 ml, dioxane 300 ml and toluene to 1000 ml: Baxter, Fanning & Swartz (1964)]. The vials were sealed with screw caps and agitated to ensure a homogeneous mixture.

The initial activity was determined by taking 0·1 ml samples of the solutions before perfusion and adding these to 10 ml amounts of the liquid scintillation solvent system. All samples were stored for 24 hr at −20° in the detector deep freeze for dark and temperature adaptation.

The activity of each sample was determined at the balance point for ^{14}C by means of a liquid scintillation detector and associate beta spectrometer (Ekco Model N664A and Scaler Model N610A). A blank sample was used to measure the background. The true net cpm for each sample

TABLE 1. INTESTINAL ABSORPTION OF SALICYLIC ACID[1]

Time interval (min)	Total absorption %		Absorption %/g bodyweight	
	$\bar{X}$	Sx	$\bar{X}$	Sx
Salicylic acid alone				
10	40·7	8·2	0·49	0·12
20	43·2	9·3	0·51	0·14
30	44·9	9·8	0·51	0·06
Salicylic acid + phenylephrine				
10	67·7	15·0	0·61	0·16
20	68·6	16·4	0·62	0·17
30	67·3	10·4	0·60	0·12
Salicylic acid + caffeine				
10	71·5	15·1	0·63	0·14
20	72·9	13·7	0·64	0·15
30	73·3	13·6	0·65	0·14

[1] Values are arithmetic means of 5 animals.

was determined and corrected for quenching by means of an internal standard of hexadecane-1-^{14}C.

The percentage absorption at each time interval for each sample was calculated from the loss of activity from the initial solution. These values are in Table 1.

Preliminary perfusion through the tygon tubing and cannula with labelled salicylic acid drug solution established that there was no loss of initial activity due to absorption or adsorption in the tubing.

Results and discussion

The intestinal absorption of salicylic acid was observed to be 40·7, 43·2 and 44·9% at time intervals of 10, 20 and 30 min. These values are lower but consistent with those reported by Shanker and his associates (1958), using chemical analysis. The radioisotope approach does appear to offer several distinct advantages. The assay procedure is simple particularly when compared to the complexity of chemical analytical methods, and offers an unlimited sensitivity and specificity. In this study millimoles of salicylic acid were evaluated with the use of low microcurie levels of activity. The specificity is illustrated by the use of the identical counting procedure to determine the absorption of salicylic acid in the presence of caffeine and phenylephrine. The latter is of particular interest as chemical analytical methods could not distinguish between phenylephrine and salicylic acid. This problem would undoubtedly be true for many drug combinations. The presence of phenylephrine or caffeine was observed to increase the intestinal absorption of salicylic acid.

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